substituted ribonucleotides, and in some embodiments, these four or five unsubstituted 5' nucleotides are deoxyribonucleotides. In other embodiments, the oligonucleotide has at least one 2'-substituted ribonucleotide at both its 3' and 5' termini, and in yet other embodiments, the oligonucleotide is composed of 2'-substituted ribonucleotides in all positions with the exception of at least four or five contiguous deoxyribonucleotide nucleotides in any interior position. Another aspect of the invention includes the administration of an oligonucleotide composed of nucleotides that are all 2'-substituted ribonucleotides. Particular embodiments include oligonucleotides having a 2'-O-alkyl-ribonucleotide such as a 2'-O methyl. Other embodiments include the administration of chimeric oligonucleotides. In one preferred embodiment, the chimeric oligonucleotide has at least one alkylphosphonate internucleotide linkage at both its 3' and 5' ends and having phosphorothioate internucleotide linkages.

In another embodiment of the invention, the oligonucleotide administered has at least one deoxyribonucleotide, and in a preferred embodiment, the oligonucleotide has at least four or five contiguous deoxyribonucleotides capable of activating RNase H.

The oligonucleotide administered is complementary to a gene of a virus, pathogenic organism, or a cellular gene in some embodiments of the invention. In some embodiments, the

oligonucleotide is complementary to a gene of a virus involved in AIDS, oral or genital herpes, papilloma warts, influenza, foot and mouth disease, yellow fever, chicken pox, shingles, adult T-cell leukemia, Burkitt's lymphoma, nasopharyngeal carcinoma, or hepatitis. In one particular embodiment, the oligonucleotide is complementary to an HIV gene and includes about 15 to 26 nucleotides linked by phosphorothioate internucleotide linkages, at least one of the nucleotides at the 3' terminus being a 2'-substituted ribonucleotide, and at least four contiguous deoxyribonucleotides.

In another embodiment, the oligonucleotide is complementary to a gene encoding a protein in associated with Alzheimer's disease.

In yet other embodiments, the oligonucleotide is complementary to a gene encoding a protein expressed in a parasite that causes a parasitic disease such as amebiasis, Chagas' disease, toxoplasmosis, pneumocytosis, giardiasis, cryptoporidiosis, trichomoniasis, malaria, ascariasis, filariasis, trichinosis, or schistosomiasis infections.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 is a graphic representation showing the time course of radiolabelled oligonucleotide in liver, kidney and plasma following the oral administration of radiolabelled phosphorothioate (PS) oligonucleotide 1 (SEQ ID NO:10);

FIG. 2A is a representation of an autoradiogram of radiolabelled oligonucleotide in the stomach, small intestine, and large intestine of rats at different times following oral administration of PS oligonucleotide;

FIG. 2B is a representation of an autoradiogram of radiolabelled oligonucleotide in the stomach, small intestine, and large intestine of rats at different times following oral administration of hybrid oligonucleotide;

FIG. 3A is a representation of an autoradiogram of radiolabelled oligonucleotide in the stomach, small intestine, and large intestine of mice at different times following oral administration of hybrid oligonucleotide;

FIG. 3B is a representation of an autoradiogram of radiolabelled oligonucleotide in the stomach, small intestine, and large intestine of mice at different times following oral administration of chimeric oligonucleotide;

FIG. 4A is an HPLC chromatograph of radiolabelled PS oligonucleotide standard;

FIG. 4B is an HPLC chromatogram of oligonucleotides extracted from plasma samples taken 12 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 5A is an HPLC chromatogram of radiolabelled PS oligonucleotide standard;

FIG. 5B is an HPLC chromatogram of oligonucleotides extracted from rat liver 6 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 5C is an HPLC chromatogram of oligonucleotides extracted from rat liver 24 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 6 is a graphic representation demonstrating the time course of urinary excretion of radioactivity in rats following the oral administration of radiolabelled PS oligonucleotide;

FIG. 7A is an HPLC chromatogram of radiolabelled PS oligonucleotide standard;

FIG. 7B is an HPLC chromatogram of oligonucleotides extracted from rat urine 6 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 7C is an HPLC chromatogram of oligonucleotides extracted from rat urine 12 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 8 is a graphic representation showing the course of radioactivity in the gastrointestinal tract and feces in rats following the oral administration of radiolabelled PS oligonucleotide;

FIG. 9 is an HPLC chromatogram of oligonucleotides extracted from rat stomach 1 hour, 3 hours, and 6 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 10 is an HPLC chromatogram of oligonucleotides extracted from rat large intestine 3 hours, 6 hours, and 12 hours after the administration of radiolabelled PS oligonucleotide;

FIG. 11A is a representation of an autoradiogram of radiolabelled oligonucleotide in the plasma, liver, kidney, spleen, heart, and lung of mice 6 hours following oral administration of hybrid oligonucleotide;

FIG. 11B is a representation of an autoradiogram of radiolabelled oligonucleotide in the plasma, liver, kidney, spleen, heart, and lung of mice 6 hours following oral administration of chimeric oligonucleotide; and

FIG. 12 is a graphic representation of the distribution of radioactivity in GI + feces, plasma, tissue, and urine at various times following oral administration of PS oligonucleotide (30 mg/kg rat), hybrid oligonucleotide (10 mg/kg mouse), and chimeric oligonucleotide (10 mg/kg mouse).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patent, allowed patent applications, and articles cited herein are hereby incorporated by reference.

This invention provides a method of down-regulating the expression of a gene in an animal by the oral administration of an oligonucleotide whose nucleotide sequence is complementary to the targeted gene.

It is known that an oligonucleotide, called an "antisense oligonucleotide," can bind to a target single-stranded nucleic acid molecule according to the Watson-Crick or the Hoogsteen rule of base pairing, and in doing so, disrupt the function of the target by one of several mechanisms: by preventing the binding of factors required for normal transcription, splicing, or translation; by triggering the enzymatic destruction of mRNA by RNase H if a contiguous region of deoxyribonucleotides exists in the oligonucleotide, and/or by destroying the target via reactive groups attached directly to the antisense oligonucleotide.

Thus, because of the properties described above, such oligonucleotides are useful therapeutically by their ability to control or down-regulate the expression of a particular gene

in an animal, according to the method of the present invention.

The oligonucleotides useful in the method of the invention are at least 6 nucleotides in length, but are preferably 6 to 50, more preferably 11 to 35, most preferably 15 to 30, and commonly 15 to 25 nucleotides in length. They are composed of deoxyribonucleotides, ribonucleotides, or a combination of both, with the 5' end of one nucleotide and the 3' end of another nucleotide being covalently linked by non-phosphodiester internucleotide linkages. Such linkages include alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. Oligonucleotides with these linkages can be prepared according to known methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer as described by Brown (*A Brief History of Oligonucleotide Synthesis. Protocols for Oligonucleotides and Analogs, Methods in Molecular Biology* (1994) **20**:1-8). (See also, e.g., Sonveaux "Protecting Groups in Oligonucleotides Synthesis" in Agrawal (1994) *Methods in Molecular Biology* **26**:1-72; Uhlmann et al. (1990) *Chem. Rev.* **90**:543-583).

The oligonucleotides of the composition may also be modified in a number of ways without compromising their ability to hybridize to the

target nucleic acid. Such modifications include, for example, those which are internal or at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the viral genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position). Other modified oligonucleotides are capped with a nuclease resistance-conferring bulky substituent at their 3' and/or 5' end(s) , or have a substitution in one nonbridging oxygen per nucleotide. Such modifications can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule. For the preparation of such modified oligonucleotides, see, e.g., Agrawal (1994) *Methods in Molecular Biology* **26**; Uhlmann et al. (1990) *Chem. Rev.* **90**:543-583).

Oligonucleotides which are self-stabilized are also considered to be modified oligonucleotides useful in the methods of the invention (Tang et al. (1993) *Nucleic Acids Res.*

20:2729-2735). These oligonucleotides comprise two regions: a target hybridizing region; and a self-complementary region having an oligonucleotide sequence complementary to a nucleic acid sequence that is within the self-stabilized oligonucleotide.

The preparation of these unmodified and modified oligonucleotides is well known in the art (reviewed in Agrawal et al. (1992) *Trends Biotechnol.* ***10**:152-158)* *(see, e.g.,* Uhlmann et al. (1990) *Chem. Rev.* **90**:543-584; and (1987) *Tetrahedron. Lett.* **28**:(31):3539-3542); Agrawal (1994) *Methods in Molecular Biology* **20**:63-80); and Zhang et al. (1996) *J. Pharmacol. Expt. Thera*. **278**:1-5).

The oligonucleotides administered to the animal may be hybrid oligonucleotides in that they contain both deoxyribonucleotides and at least one 2′ substituted ribonucleotide. For purposes of the invention, the term "2′-substituted" means substitution at the 2′ position of the ribose with, e.g., a -O-lower alkyl containing 1-6 carbon atoms, aryl or substituted aryl or allyl having 2-6 carbon atoms e.g., 2′-O-allyl, 2′-O-aryl, 2′-O-alkyl, 2′-halo, or 2′-amino, but not with 2′-H, wherein allyl, aryl, or alkyl groups may be unsubstituted or substituted, e.g., with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl or amino groups. Useful substituted ribonucleotides are 2′-0-alkyls such as 2′-0-methyl.

The hybrid DNA/RNA oligonucleotides useful in the method of the invention resist nucleolytic degradation, form stable duplexes with RNA or DNA, and preferably activate RNase H when hybridized with RNA. They may additionally include at least one unsubstituted ribonucleotide. For example, an oligonucleotide useful in the method of the invention may contain all deoxyribonucleotides with the exception of one 2′ substituted ribonucleotide at the 3′ terminus of the oligonucleotide. Alternatively, the oligonucleotide may have at least one substituted ribonucleotide at both its 3′ and 5′ termini.

One preferred class of oligonucleotides useful in the method of the invention contains four or more deoxyribonucleotides in a contiguous block, so as to provide an activating segment for RNase H. In certain cases, more than one such activating segment will be present at any location within the oligonucleotide. There may be a majority of deoxyribonucleotides in oligonucleotides according to the invention. In fact, such oligonucleotides may have as many as all but one nucleotide being deoxyribonucleotides. Thus, a preferred oligonucleotide having from about 2 to about 50 nucleotides or most preferably from about 12 to about 25 nucleotides, the number of deoxyribonucleotides present ranges from 1 to about 24. Other useful oligonucleotides may consist only of 2′-substituted ribonucleotides.

TABLE 1 lists some representative species of oligonucleotides which are useful in the method of

the invention. 2'-substituted nucleotides are underscored.

TABLE 1

OLIGO NO.	OLIGONUCLEOTIDE	SEQ ID NO.:
1	CTCTCGCACCCATCTCTCTCCTTCU	1
2	CTCTCGCACCCATCTCTCTCCTUCU	2
3	CTCTCGCACCCATCTCTCTCCUUCU	3
4	CTCTCGCACCCATCTCUCUCCUUCU	4
5	CTCTCGCACCCAUCUCUCUCCUUCU	5
6	CTCTCGCACCCAUCUCUCUCCUUCU	6
7	CTCTCGCACCCAUCUCUCUCCUUCU	7
8	CUCUCGCACCCAUCUCUCUCCUUCU	8
9	CTCTCGCACCCATCTCTCTCCTTCU	1
10	CUCTCGCACCCATCTCTCTCCTTCU	9
11	CUCUCGCACCCATCTCTCTCCUUCU	10
12	CUCUCGCACCCATCTCUCUCCUUCU	11
13	CUCUCGCACCCAUCUCUCUCCUUCU	12
14	CUCUCGCACCCATCTCTCUCCUUCU	13
15	CTCTCGCACCCAUCUCUCUCCUUCU	5
16	CUCUCGCACCCAUCTCTCTCCUUCU	14
17	CUCUCGCACCCATCTCTCTCCUUCU	15
18	CUCTCGCACCCAUCUCUCUCCUUCU	16
19	CUCTCGCACCCATCTCTCUCCUUCU	17

The oligonucleotides administered to the animal may be chimeric in that they contain more than one type of internucleotide linkage. Such chimeric oligonucleotides are described in U.S. Patent Nos. 5,149,797 and 5,366,878. For example, chimeric oligonucleotides useful in the method of the invention may include phosphorothioate and alkylphosphonate internucleotide linkages. One preferred alkylphosphonate linkage is a methylphosphonate linkage.

Table 2 lists some representative specifics of chimeric oligonucleotides which are useful in the method of the invention. The alkylphosphonate internucleotide linkages are indicated by ":"; the phosphorothioate linkages are indicated by "-".

TABLE 2

NO:	OLIGONUCLEOTIDE (5'→3')	SEQ ID NO:
20	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T:T:C:T	18
21	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T:T:C:T	18
22	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T:T:C:T	18
23	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T:T:C:T	18
24	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T:T:C:T	18
25	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T-T:C:T	18
26	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T-T:C:T	18
27	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T-T:C:T	18
28	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T:T:C:T	18
29	C:T-C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T:T:C:T	18
30	C:T-C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T:T:C:T	18
31	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T-T-C:T	18
32	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T-T-C:T	18

TABLE 2 (CON'T)

NO:	OLIGONUCLEOTIDE (5'→3')	SEQ ID NO:
33	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T	19
34	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
35	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T	19
36	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T	19
37	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
38	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
39	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
40	C:T:C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
41	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
42	C:T-C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C:T	19
43	C:T-C-T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
44	C:T:C:T:C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19
45	C:T:C:T-C-G-C-A-C-C-C-A-T-C-T-C-T-C-T-C-C-T	19

The oligonucleotides according to the invention are effective in inhibiting the expression of various genes in viruses, pathogenic organisms, or in inhibiting the expression of cellular genes. The ability to inhibit such agents is clearly important to the treatment of a variety of disease states. Thus, oligonucleotides according to the method of the invention have a nucleotide sequence which is complementary to a nucleic acid sequence that is from a virus, a pathogenic organism or a cellular gene. Preferably such oligonucleotides are from about 6 to about 50 nucleotides in length.

For purposes of the invention, the term "oligonucleotide sequence that is complementary to a nucleic acid sequence" is intended to mean an oligonucleotide sequence that binds to the target nucleic acid sequence under physiological conditions, e.g., by Watson-Crick base pairing (interaction between oligonucleotide and single-stranded nucleic acid) or by Hoogsteen base pairing (interaction between oligonucleotide and double-stranded nucleic acid) or by any other means including in the case of a oligonucleotide binding to RNA, pseudoknot formation. Such binding (by Watson Crick base pairing) under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence.

The nucleic acid sequence to which an oligonucleotide according to the invention is complementary will vary, depending upon the gene

to be down-regulated. In some cases, the target gene or nucleic acid sequence will be a virus nucleic acid sequence. The use of antisense oligonucleotides to inhibit various viruses is well known (reviewed in Agrawal (1992) *Trends in Biotech.* **10:**152-158). Viral nucleic acid sequences that are complementary to effective antisense oligonucleotides have been described for many viruses, including human immunodeficiency virus type 1 (HIV-1) (U.S. Patent No. 4,806,463), herpes simplex virus (U.S. Patent No. 4,689,320), influenza virus (U.S. Patent No. 5,194,428), and human papilloma virus (Storey et al. (1991) *Nucleic Acids Res.* **19:**4109-4114). Sequences complementary to any of these nucleic acid sequences can be used for oligonucleotides according to the invention, as can be oligonucleotide sequences complementary to nucleic acid sequences from any other virus. Additional viruses that have known nucleic acid sequences against which antisense oligonucleotides can be prepared include, but are not limited to, foot and mouth disease virus (see, Robertson et al. (1985) *J. Virol.* **54:**651; Harris et al. *(1980) Virol.* **36:**659), yellow fever virus (see Rice et al. (1985) *Science* **229:**726), varicella-zoster virus (see, Davison and Scott (1986) *J. Gen. Virol.* **67:**2279), Epstein-Barr virus, cytomegalovirus, respiratory syncytial virus (RSV), and cucumber mosaic virus (see Richards et al. (1978) *Virol.* **89:**395).

For example, an oligonucleotide has been designed which is complementary to a portion of the HIV-1 gene, and as such, has significant anti-HIV effects (Agrawal (1992) *Antisense Res. Development* **2**:261-266). The target of this oligonucleotide has been found to be conserved among various HIV-1 isolates. It is 56% G + C rich, water soluble, and relatively stable under physiological conditions. This oligonucleotide binds to a complementary RNA target under physiological conditions, with the T of the duplex approximately being 56°C. The antiviral activity of this oligonucleotide has been tested in several models, including acutely and chronically infected CEM cells, long-term cultures mimicking *in vivo* conditions, human peripheral blood lymphocytes and macrophages, and isolates from HIV-1 infected patients (Lisziewicz et al. (*Proc. Natl. Acad. Sci. (USA)* (1992) **89**:11209-11213); Lisziewicz et al. (*Proc. Natl. Acad. Sci. (USA)* (1993) **90**:3860-3864); Lisziewicz et al. (*Proc. Natl. Acad. Sci. (USA)* (1994) **91**:7942-7946); Agrawal et al. (*J. Ther. Biotech*) in press).

The oligonucleotides according to the invention alternatively can have an oligonucleotide sequence complementary to a nucleic acid sequence of a pathogenic organism. The nucleic acid sequences of many pathogenic organisms have been described, including the malaria organism, *Plasmodium falciparum*, and many pathogenic bacteria. Oligonucleotide sequences complementary to nucleic acid sequences from any such pathogenic organism can be used in

oligonucleotides according to the invention. Nonlimiting examples of pathogenic eucaryotes having known nucleic acid sequences against which antisense oligonucleotides can be prepared include *Trypanosom abrucei gambiense* and *Leishmania* (See Campbell et al., *Nature* **311:**350 (1984)), and *Fasciola hepatica* (See Zurita et al., *Proc. Natl. Acad. Sci. USA* **84:**2340 (1987).

Antifungal oligonucleotides can be prepared using a target hybridizing region having an oligonucleotide sequence that is complementary to a nucleic acid sequence from, e.g., the chitin synthetase gene, and antibacterial oligonucleotides can be prepared using, e.g., the alanine racemase gene. Among fungal diseases that may be treatable by the method of treatment according to the invention are candidiasis, histoplasmosis, cryptococcocis, blastomycosis, aspergillosis, sporotrichosis, chromomycosis, dermatophytosis, and coccidioidomycosis. The method might also be used to treat rickettsial diseases (e.g., typhus, Rocky Mountain spotted fever), as well as sexually transmitted diseases caused by Chlamydia trachomatis or Lymphogranuloma venereum. A variety of parasitic diseases may be treated by the method according to the invention, including amebiasis, Chagas' disease, toxoplasmosis, pneumocystosis, giardiasis, cryptosporidiosis, trichomoniasis, and Pneumocystis carini pneumonia; also worm (helminthic) diseases such as ascariasis, filariasis, trichinosis, schistosomiasis and nematode or cestode infections. Malaria may be

treated by the method of treatment of the invention regardless of whether it is caused by P. falciparum, P. vivas, P. orale, or P. malariae.

The infectious diseases identified above may all be treated by the method of treatment according to the invention because the infectious agents for these diseases are known and thus oligonucleotides according to the invention can be prepared, having oligonucleotide sequence that is complementary to a nucleic acid sequence that is an essential nucleic acid sequence for the propagation of the infectious agent, such as an essential gene.

Other disease states or conditions that may be treatable by the method according to the invention are those which result from an abnormal expression or product of a cellular gene. These conditions may be treated by administration of oligonucleotides according to the invention, and have been discussed earlier in this disclosure.

Other oligonucleotides according to the invention can have a nucleotide sequence complementary to a cellular gene or gene transcript, the abnormal expression or product of which results in a disease state. The nucleic acid sequences of several such cellular genes have been described, including prion protein (Stahl et al. (1991) *FASEB J.* **5**:2799-2807), the amyloid-like protein associated with Alzheimer's disease (U.S. Patent No. 5,015,570), and various well-known oncogenes and proto-oncogenes, such as c-*myb*, c-

myc, c-*abl*, and n-*ras*. In addition, oligonucleotides that inhibit the synthesis of structural proteins or enzymes involved largely or exclusively in spermatogenesis, sperm motility, the binding of the sperm to the egg or any other step affecting sperm viability may be used as contraceptives. Similarly, contraceptives for women may be oligonucleotides that inhibit proteins or enzymes involved in ovulation, fertilization, implantation or in the biosynthesis of hormones involved in those processes.

Hypertension may be controlled by oligonucleotides that down-regulate the synthesis of angiotensin converting enzyme or related enzymes in the renin/angiotensin system. Platelet aggregation may be controlled by suppression of the synthesis of enzymes necessary for the synthesis of thromboxane A2 for use in myocardial and cerebral circulatory disorders, infarcts, arteriosclerosis, embolism and thrombosis. Deposition of cholesterol in arterial wall may be inhibited by suppression of the synthesis of fatty acid co-enzyme A: cholesterol acyl transferase in arteriosclerosis. Inhibition of the synthesis of cholinephosphotransferase may be useful in hypolipidemia.

There are numerous neural disorders in which hybridization arrest may be used to reduce or eliminate adverse effects of the disorder. For example, suppression of the synthesis of monoamine oxidase may be used in Parkinson's disease. Suppression of catechol o-methyl transferase may

be used to treat depression; and suppression of indole N-methyl transferase may be used in treating schizophrenia.

Suppression of selected enzymes in the arachidonic acid cascade which leads to prostaglandins and leukotrienes may be useful in the control of platelet aggregation, allergy, inflammation, pain and asthma.

Suppression of the protein expressed by the multidrug resistance (*mdr*-1) gene, which can be responsible for development of resistance of tumors to a variety of anti-cancer drugs and is a major impediment in chemotherapy may prove to be beneficial in the treatment of cancer. Oligonucleotide sequences complementary to nucleic acid sequences from any of these genes can be used for oligonucleotides according to the invention, as can be oligonucleotide sequences complementary to any other cellular gene transcript, the abnormal expression or product of which results in a disease state.

The oligonucleotides described herein are administered orally or enterally to the animal subject in the form of therapeutic pharmaceutical formulations that are effective for treating virus infection, infections by pathogenic organisms, or disease resulting from abnormal gene expression or from the expression of an abnormal gene product. In some aspects or the method according to the invention, the oligonucleotides are administered

in conjunction with other therapeutic agents, e.g., AZT in the case of AIDS.

The therapeutic pharmaceutical formulation containing the oligonucleotide includes a physiologically acceptable carrier, such as an inert diluent or an assimilable edible carrier with which the peptide is administered. Suitable formulations that include pharmaceutically acceptable excipients for introducing compounds to the bloodstream by other than injection routes can be found in *Remington's Pharmaceutical Sciences* (18th ed.) (Genarro, ed. (1990) Mack Publishing Co., Easton, PA). The oligonucleotide and other ingredients may be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. The oligonucleotide may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. When the oligonucleotide is administered orally, it may be mixed with other food forms and pharmaceutically acceptable flavor enhancers. When the oligonucleotide is administered enterally, they may be introduced in a solid, semi-solid, suspension, or emulsion form and may be compounded with any number of well-known, pharmaceutically acceptable additives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated such as those described in U.S. Patent Nos. 4,704,295, 4,556,552, 4,309,404, and 4,309,406.

The amount of oligonucleotide in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 50 micrograms to about 200 mg per kg body weight of the animal, with 10 mg to 100 mg per kg being most preferable.

It will be appreciated that the unit content of active ingredient or ingredients contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units (such as capsules or tablets or combinations thereof).

In order to determine if the oligonucleotide administered according to the method of the invention is absorbed into body tissues, and if so, in which tissues absorption occurs, the following study was performed. Samples of various body tissues from treated rats were analyzed for radioactivity at increasing hours after oral administration of a radioactively labelled phosphorothioate oligonucleotide. FIG. 1 illustrates the plasma, liver, and kidney concentration-time course of an oligonucleotide equivalents after oral administration of the radiolabelled oligonucleotide. These results demonstrate that the drug is absorbed through

gastrointestinal tract and accumulated in the kidney and the liver.

As illustrated in FIGS. 2A and 2B, both unmodified and hybrid oligonucleotides were shown to be stable in the stomach up to 6 hr following oral administration. The unmodified oligonucleotide underwent extensive degradation in small and large intestine, the majority of the radioactivity being associated with the different length of truncated oligonucleotide (FIG. 2A). In contrast, the hybrid oligonucleotide was more stable compared to the unmodified oligonucleotide, the majority of the radioactivity in small intestine being associated with the intact oligonucleotide (FIG. 2B). Increased degradation of the hybrid oligonucleotide was observed in the large intestine (FIG. 2B).

^{35}S-labelled modified oligonucleotides were also orally administered to mice at a single dose. For the hybrid oligonucleotide, similar profiles of gel electrophoresis of radioactivity in the gastrointestinal tract were observed with mice compared to rats (FIG. 3A). For the chimeric oligonucleotide, gel electrophoresis of radioactivity in the gastrointestinal tract revealed that this compound was stable in stomach and small intestine, with significant degradation in large intestine (FIG. 3B).

The chemical form of radioactivity in rat plasma was further evaluated by HPLC as shown is FIG. 4A and 4B, demonstrating the presence of both

intact PS oligonucleotide (A) as well as metabolites (B) 12 hours after oral administration (see FIG. 4B). Intact oligonucleotide was also detected in rat liver 6 hours (FIG. 5B) and 12 hours (FIG. 5C) after oral administration. Radioactivity in rat brain, thymus, heart, lung, liver, kidney, adrenals, stomach, small intestine, large intestine, skeletal muscle, testes, thyroid, epidermis, whole eye, and bone marrow was detectable 48 hours after oral administration of the radiolabelled oligonucleotide. For unmodified oligonucleotide, minimal intact form was detectable in rat tissue samples. However, as shown in FIG. 11A for the hybrid oligonucleotide and in FIG. 11B for the chimeric oligonucleotide, intact oligonucleotides were detected in plasma and tissue samples of the liver, kidney, spleen, heart, and lung.

Further evidence to support the absorption of the oligonucleotide comes from urine sample analysis after radioactively labelled oligonucleotide was orally administered. FIG. 6 shows the cumulative excretion of the radioactively labelled oligonucleotide into the urine over 48 hr following the administration of radiolabelled phosphorothioate oligonucleotide. That the oligonucleotide continues to be excreted in the urine over time implies that other tissues had absorbed it, and that the body was capable of absorption for an extended period of time. FIGS. 7B and 7C demonstrate that although the majority of radioactivity in urine was present as degradative products, intact oligonucleotide was

also detected, demonstrating that this oligonucleotide is absorbed intact.

To determine the level of bioavailability of oligonucleotides following oral administration, the level of the oligonucleotide in the gastrointestinal tract (stomach and intestine) and feces was measured. FIG. 8 shows that approximately 80% of administered oligonucleotide remained or was excreted in feces, indicating that 20% of administered oligonucleotide was absorbed. This oligonucleotide was stable in stomach; no significant degradative products in stomach contents were detected six hours after oral administration (FIG. 9). The majority of administered oligonucleotide in the contents of the large intestine were also present as the intact compound (FIG. 10).

In another study, the oral bioavailability of unmodified, hybrid, and chimeric oligonucleotide administered to rat and mouse were compared, based on the quantitation of radioactivity in the gastrointestinal tract, feces, plasma, urine and remaining tissues at various times. Total recovery of radioactivity in the study was 92 ± 6%. The total absorption of unmodified oligonucleotide was shown to be 17.3 ± 5.5% over 6 hr and 35.5 ± 6.0% over 12 hr following oral administration of the radiolabelled unmodified oligonucleotide to rats at a dose of 30 mg/kg. Minimal intact unmodified oligonucleotide was also detected in tissues outside enterohepatic system.

The total absorption of hybrid oligonucleotide was determined to be 10.2 ± 2.5% over 6 hr and 25.9 ± 4.7% over 12 hr following oral administration of the radiolabelled hybrid oligonucleotide in rats. Although the total absorption rates were slightly lower than that of the PS oligonucleotide, the hybrid oligonucleotide-derived radioactivity was stable in various tissues. The total absorption of the chimeric oligonucleotide was determined to be 23.6 ± 2.8% over 6 hr and 39.3 ± 2.4% over 12 hr following oral administration of the radiolabelled oligonucleotide. The comparison of oral availability of the three types of oligonucleotides is shown in FIG. 12, expressed as the percentages of administered doses in the gastrointestinal tract plus feces, in plasma, in tissues, and in urine.

Oral absorption of oligonucleotides in fasting animals was also determined with PS-oligonucleotide and hybrid oligonucleotide. Decreased absorption rates were found, indicating that the retention time of the oligonucleotides in the gastrointestinal tract in the fasting animals may be lower that in non-fasting animals.

These studies indicate that hybrid and chimeric oligonucleotides have enhanced bioavailability, which is associated with their stability in the gastrointestinal tract and other tissues.

Thus, using the method of the invention, successful absorption of oligonucleotides was accomplished through the gastrointestinal tract and distributed throughout the body. Intact oligonucleotides were detected in plasma and various tissues and excreted into the urine. These results demonstrate that oral administration is a potential means for delivery of oligonucleotides as therapeutic agents.

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLES

1. Synthesis and Analysis of Oligonucleotides

An unmodified HIV-specific 25mer oligonucleotide and hybrid 25mer phosphorothioate-linked oligonucleotide having SEQ ID NO:10 and containing 2'-0-methyl ribonucleotide 3' and 5' sequences and a deoxyribonucleotide interior, as well as two hybrid 18mer phosphorothioate-linked oligonucleotides having SEQ ID NOS:20 and 21, and containing 2'-0-methyl ribonucleotide 3' and 5' sequences and a deoxyribonucleotide interior, were synthesized, purified, and analyzed as follows.

Unmodified phosphorothioate deoxynucleosides were synthesized on CPG on a 5-6 μmole scale on an automated synthesizer (model 8700, Millipore, Bedford, MA) using the H-phosphonate approach described in U.S. Patent No. 5,149,798. Deoxynucleoside H-phosphonates were obtained from Millipore (Bedford, MA). 2'-O-methyl ribonucleotide H-phosphonates or phosphorothioates were synthesized by standard procedures (see, e.g., "Protocols for Oligonucleotides and Analogs" in *Meth. Mol. Biol.* (1993) volume 20) or commercially obtained (e.g., from Glenn Research, Sterling, VA and Clontech, Palo Alto, CA). Segments of oligonucleotides containing 2'-O-methyl nucleoside(s) were assembled by using 2'-O-methyl ribonucleoside H-phosphonates or phosphorothioates for the desired cycles. Similarly, segments of oligonucleotides containing deoxyribonucleosides were assembled by using deoxynucleoside H-phosphonates for the desired cycles. After assembly, CPG bound oligonucleotide H-phosphonate was oxidized with sulfur to generate the phosphorothioate linkage. Oligonucleotides were then deprotected in concentrated NH_4OH at 40°C for 48 hours.

Crude oligonucleotide (about 500 A_{260} units) was analyzed on reverse low pressure chromatography on a C_{18} reversed phase medium. The DMT group was removed by treatment with 80% aqueous acetic acid, then the oligonucleotides were dialyzed against distilled water and lyophilized.

Chimeric oligonucleotide was prepared as described inZhang et al. (*J. Pharmacol. Exptal. Thera.* (1996) **278**:(in press)). This chimeric oligonucleotide had 3 methylphosphate internucleotide linkages at its 5' end, 4 methylphosphonate internucleotide linkages at its 3' end, and phosphorothioate internucleotide linkages elsewhere in the molecule were prepared and purified as follows. The first four couplings were carried out by using nucleoside methyl-phosphoramidite, followed by oxidation with a standard iodine reagent. The next seven couplings were carried out by using nucleoside β-cyanoethylphosphoramidite, followed by oxidation with 3H-1,2-benzodithiole-3-one-1,1,-dioxide. The eighth coupling was carried out by using nucleoside β-cyanoethylphosphoramidite. After several washes with acetonitrile, the column was removed from the machine, and CPG-bound oligonucleotide was removed from the column and placed in an Eppendorf tube (1.5 ml).

2. Radioactive Labelling of Oligonucleotide

To obtain ^{35}S-labelled oligonucleotide, synthesis was carried out in two steps. The first 19 nucleotides of the sequence SEQ ID NO:1) from its 3'-end were assembled using the β-cyanoethyl-phosphoramidite approach (see, Beaucage in *Protocols for Oligonucleotides and Analogs* (Agrawal, ed.), Humana Press, (1993), pp. 33-61). The last six nucleotides were assembled using the H-phosphonate approach (see, Froehler in *Protocols for Oligonucleotides and Analogs* (Agrawal, ed.) Humana Press, 1993, pp.

10 min. The supernatant was removed and the CPG-bound oligonucleotide was washed with CH_3CN (10 x 1 ml). After capping with acetic anhydride (300 μl, tetrahydrofuran-lutidine-acetic anhydride, 8:1:1) and dimethylaminopyridine (300 μl, 0.625% in pyridine), the ^{35}S-CPG-bound oligonucleotide was washed with acetonitrile (10 x 1 ml) and packed in the column. For the next eight couplings, we used nucleoside β-cyanoethylphosphoramidite followed by oxidation with 3H-1,2-benzodithiole-3-one-1,1-dioxide. The last four couplings were carried out by using nucleoside methylphosphonamidite followed by oxidation with iodine reagent. The crude CPG-bound 25mer chimeric oligonucleotide was treated with concentrated ammonium hydroxide (28%, 3 ml) at 25°C for 2 hr. Evaporation on a Speed-Vac concentrator yielded a dried yellow pellet as crude ^{35}S-labelled chimeric PS-oligonucleotide, which was immediately treated with a solution of ethylenediamine-ethanol-water (50:45:5, v/v/v/, 4 ml) for 4.5 hr at 25°C. Purification by PAGE (20% polyacrylamide, 7 M urea) gave pure ^{35}S-labeled chimeric oligonucleotide as a white pellet (194 A_{260} units, 155 μCi, 180 μCi/mol). Other chemicals and reagents used in the present study were of the highest grade available.

3. Animals and Treatment

Male Sprague-Dawley rats (110 +/- 10 g, Harlan Laboratories, Indianapolis, IN) and male CD-/F2 mice (25 ± 3 g, Charles River Laboratory, Wilmington, MA) were used in the study. The animals were fed with commercial diet and water *ad*

libitum for 1 week prior to the study.

Unlabelled and ^{35}S-labelled oligonucleotides were dissolved in physiological saline (0.9% NaCl) in a concentration of 25 mg/ml, and were administered to the fasted animals via gavage at the designated dose (30-50 mg/kg for rats and 10 mg/kg for mice). Doses were based on the pretreatment body weight and rounded to the nearest 0.01 ml. After dosing, each animal was placed in a metabolism cage and fed with commercial diet and water *ad libitum*. Total voided urine was collected and each metabolism cage was then washed following the collection intervals. Total excreted feces was collected from each animal at various timepoints, and feces samples were homogenized prior to quantitation of radioactivity. Blood samples were collected in heparinized tubes from animals at the various timepoints. Plasma was separated by centrifugation. Animals were euthanized by exsanguination under sodium pentobarbital anesthesia at various times (i.e., 1, 3, 6, 12, 24, and 48 hr; 3 animals/time point). Following euthanasia, the tissues were collected from each animal. All tissues/organs were trimmed of extraneous fat or connective tissue, emptied and cleaned of all contents, individually weighed, and the weights recorded prior to homogenization.

To quantitate the total absorption of the hybrid oligonucleotide, two additional groups of animals (3 per group) for each test oligonucleotide were treated using the same

procedure as above. Animals were killed at 6 or 12 hr post dosing, and the gastrointestinal tract was then removed. Radioactivities in the gastrointestinal tract, feces, urine, plasma, and the remainder of the body were determined separately. Total recovery of radioactivity was also determined to be 95 ± 6%. The percentage of the absorbed hybrid oligonucleotide-derived radioactivity was determined by the following calculation:

$$\frac{\text{(total radioactivity in the remainder of the body + total radioactivity in urine)}}{\text{(total radioactivity in the gastrointestinal tract, feces, urine, plasma, and the remainder of the body)}} \times 100\%$$

4. Total Radioactivity Measurements

The total radioactivities in tissues and body fluids were determined by liquid scintillation spectrometry (LS 6000TA, Beckman, Irvine, CA). In brief, biological fluids (plasma, 50-100 μl; urine, 50-100 μl) were mixed with 6 ml scintillation solvent (Budget-Solve, RPI, Mt. Prospect, IL) to determine total radioactivity. Feces were ground and weighed prior to being homogenized in a 9-fold volume of 0.9% NaCl saline. An aliquot of the homogenate (100 μl) was mixed with solubilizer (TS-2, RPI, Mt. Prospect, IL) and then with scintillation solvent (6 ml) to permit quantitation of total radioactivity.

Following their removal, tissues were immediately blotted on Whatman No. 1 filter paper and weighed prior to being homogenized in 0.9% NaCl saline (3-5 ml per gram of wet weight). The resulting homogenate (100 μl) was mixed with solubilizer (TS-2, RPI, Mt. Prospect, IL) and then with scintillation solvent (6 ml) to determine total radioactivity. The volume of 0.9% NaCl saline added to each tissue sample was recorded. The homogenized tissues/organs were kept frozen at $\leq$-70°C until the use for further analysis.

5. <u>HPLC Analysis</u>

The radioactivity in urine was analyzed by paired-ion HPLC using a modification of the method described essentially by Sands et al. (*Mol. Pharm.* (1994) **45**:932-943). Urine samples were centrifuged and passed through a 0.2-μm Acro filter (Gelman, Ann Arbor, MI) prior to analysis. Hybrid oligonucleotide and metabolites in plasma samples were extracted using the above methods in sample preparation for PAGE. A Microsorb MV-C4 column (Rainin Instruments, Woburn, MA) was employed in HPLC using a Hewlett Packard 1050 HPLC with a quaternary pump for gradient making. Mobile phase included two buffers; Buffer A was 5 mM-A reagent (Waters Co., Bedford, MA) in water and Buffer B was 4:1 (v/v) Acetonitrile (Fisher)/water. The column was eluted at a flow rate of 1.5 ml/min, using the following gradient: (1) 0-4 min, 0% buffer B; (2) 4-15 min 0-35% Buffer B; and (3) 15-70 min 35%-80% Buffer B. The column was equilibrated with Buffer A for at least

30 min prior to the next run. By using a RediFrac fraction collector (Pharmacia LKB Biotechnology, Piscataway, NJ), 1-min fractions (1.5 ml) were collected into 7-ml scintillation vials and mixed with 5 ml scintillation solvent to determine radioactivity in each fraction.

6. PAGE and Autoradiography

Plasma and tissue homogenates were incubated with proteinase K (2 mg/ml) in extraction buffer (0.5% SDS/10 mM NaCl/20 mM Tris-HCl, pH 7.6/10 mM EDTA) for 1 hr at 60°C. The samples were then extracted twice with phenol/chloroform (1:1, v/v) and once with chloroform. After ethanol precipitation, the extracts were analyzed by electrophoresis in 20% polyacrylamide gels containing 7 M urea. Urine samples were filtered, desalted and then analyzed by polyacrylamide gel electrophoresis (PAGE). The gels were fixed in 10% acetic acid/10% methanol solution and then dried before autoradiography.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Agrawal, Sudhir
Diasio, Robert H.
Zhang, Ruiwen

(ii) TITLE OF INVENTION: A METHOD OF DOWN-REGULATING GENE EXPRESSION

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 60 State Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/328,520
(B) FILING DATE: 25-OCT-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: HYZ-030CIP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-526-6000
(B) TELEFAX: 617-526-5000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCTCGCACC CATCTCTCTC CTTCU 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTCTCGCACC CATCTCTCTC CTUCU 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTCGCACC CATCTCTCTC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCTCGCACC CATCTCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCTCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCTCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCTCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CUCUCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CUCTCGCACC CATCTCTCTC CTTCU 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CUCUCGCACC CATCTCTCTC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CUCUCGCACC CATCTCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CUCUCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CUCUCGCACC CATCTCTCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CUCUCGCACC CAUCTCTCTC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CUCUCGCACC CATCTCTCTC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CUCTCGCACC CAUCUCUCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CUCTCGCACC CATCTCTCUC CUUCU 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCGCACC CATCTCTCTC CTTCT 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTCTCGCACC CATCTCTCTC CT 22